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# Exploration of physicochemical and phytochemical potential of *Moringa oleifera* Lam (Sehjana) fruits/pods

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## ABSTRACT

**Background:** The physicochemical evaluation is an important parameter in detecting adulteration or improper handling, therefore, physicochemical and phytochemical standardization is considered a prerequisite for the assessment of biological activity and determination of biological standards of the plant material, and it provides the analytical characteristics which may prove to be useful in fixing the physicochemical standard for herbal drugs. So, it becomes imperative to standardize the drugs to ensure their identity, quality and purity to ascertain therapeutic efficacy of herbal drugs. **Objective:** Therefore, the present study was aimed to evaluate the physicochemical and phytochemical standardization and quality control check of an important drug Sehjana (*Moringa* oleifera Lam) used for various diseases, **Material and Methods:** The test drugs, Sehjana (*Moringa oleifera* Lam.) were collected directly from the herbal garden of department of Ilmul Advia AMU, Aligarh. Which includes parameters recommended by National Unani Pharmacopeia Committee, Qualitative analysis and chromatographic studies (TLC) were performed for proper identification and quality control these parameter include **Results:** Ash values, Total ash, (6.44%) acid insoluble ash, (3.94%) water soluble ash, (2.53%) Successive extractive values in different solvent; petroleum ether (3.03%), diethyl ether (1.3%), chloroform (1.0%), acetone (1.5%), alcohol (13.1%), aqueous (18.56%), solubility in alcohol (15.2%) and water (22.93%), loss on drying (9.13%), pH at 1% (5.45), & 10% (4.81), bulk density (0.33%) and moisture content (8.6%). **Conclusion:** Preliminary phytochemical analysis of Sehjana (*Moringa oleifera* Lam.) showed presence of alkaloid, steroids, phenol, amino acid, and terpenoids, carbohydrate and protein, which may be active compound, responsible for its wide activities.

**Keywords:** Standardization, Physicochemical, Phytochemical, Sehjana, TLC,

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## INTRODUCTION

Herbal or natural drugs show significant variation in the chemical composition. This can be so drastic as to cause therapy failure or toxicity, so it can be appreciated that different samples of the same natural drug would rather commonly produce significantly different responses. So it is necessary to determine some crucial physicochemical characters of each sample before its pharmacological study to ensure that subsequent study would use same natural drugs. Therefore, along with the pharmacological study, the test drug was also subjected to a physicochemical study, the evaluation of their ash value, extractive value, and qualitative analysis is of great significance. Sehjana **(***Moringa oleifera* L.**)** has been in use since times immemorial to treat wide range of indications. Present study deals with physicochemical and phytochemical investigation of dried fruit of Sehjana. *Moringa oleifera* Lam. (Moringaceae) commonly known as Sehjana in Unani Medicine and drumstick, and horse radish tree in English. It is medium sized tree about 10-12m height[1,2]. Moringa can withstand both severe drought and mild frost conditions and hence widely cultivated across the world. Sehjana is a famous Indian drug used in a number of pathological conditions although, the entire plant has medicinal value but its fruit, leaves, seeds, have medicinal values of high importance. Its different parts are used after little processing as single drugs. The leaves are rich in protein, minerals, vitamins, carotene and antioxidant compounds, and other essential phytochemicals. Seed kernels contain a significant amount of oil (up to 40%) with a high-quality fatty acid composition (oleic acid

>70%) and after refining a notable resistance to oxidative degradation[3]. The seed pods (fruits) of the *Moringa oleifera* tree are the most nutritive and useful parts of this plant. The durable, drought-resistant nature of the moringa tree makes it a valuable source of nutrition in regions where water is scarce. Moringa seed pods are used to treat drinking water supplies also, due to their natural coagulant properties which allow particulates to settle in turbid water. The fruits of this plant are 20-45 cm long, green when young or light brown when ripe, taste is slight bitter without any definite smell[4,5]. The *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, ß-sitosterol, caffeolquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional value, *M. oleifera* is very important for its medicinal value. Various parts of this plant act as cardiac and circulatory stimulants,possess antitumour, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic[6], antihypertensive, cholesterol lowering[7], and antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities[1,5], aphrodisiac, anthelmintic, analgesic activities[8,9], Rubefacient, Vesicant[10]. According to Unani literature it possess many actions like Moohallil-e-Waram, Muqawwi-e-Bah, Mushtahi, Qatile Kiram-e-Amaa[11,12], so medicinally used in Waja-ul-Mafasil, Waja-ul-Qutu, Zof-e-Ishteha[13,14]. Botanically known as *Moringa oleifera Lam* or *Moringa Pterygosperma, Gaertn* (Moringaceae), is a fast growing softwood tree indigenous to sub-Himalayan tracts of Northern India[1]. It is one of 13 species within the same genus, and has become the most diffuse in tropical and subtropical areas at altitudes up to 2000 m[15]. It has been grown and naturalized in other countries like Pakistan, Afghanistan, Sri Lanka, Bangladesh, East and West Africa, throughout West Indies[16]. Now a days, *Moringa oleifera* and its derivative are distributed mainly in Middle East, African and Asian countries and are still spreading to others.

## MATERIALS AND METHODS

The test drugs, Sehjana (*Moringa oleifera* Lam.) were collected directly from the herbal garden of department of Ilmul Advia AMU, Aligarh. And are properly identified according to the botanical, Unani and Ayurvedic literature and then confirmed in pharmacognosy section of department of Ilmul Advia. A herbarium sample of the test drugs were prepared and submitted to mawalid-e-salasa museum of the department after identification for further reference, Sehjana Voucher no, SC- 0185/15. The drug was cleaned from the earthy material, washed with double distilled water and dried at 45 °C in hot air oven to powdered in electrical grinder with slow and light movement to avoid sticking of the drug material with the grinder and there after the drug was passed through the sieve no. 80 to confirm its fineness and uniformity of particle size. Finally the powder was stored in air tight container for experimental study.

### Physicochemical Studies

The Physicochemical study include the determination of extractive values of the test drug in different solvents, alcohol and water soluble contents, moisture content, ash value, loss of weight on drying, bulk density and pH values.

### Ash Value Determination

### Total ash

About 2 to 3 gm accurately weighed powdered drug was incinerated in silica crucible (previously ignited and weighted) at a temperature not exceeding dull red heat (450°C) in muffle furnace until free from carbon. The crucible was cooled in dessicator and weighted. The percentage of total ash was calculated with reference to air dried drug[17,18,19].

### Water soluble Ash

The ash was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on ash less filter paper (Whatmann Filter paper No.42). It was washed with hot water and was incinerated along with filter paper in a previously weighted silica crucible at a temperature not exceeding 450°C to a constant weight. The weight of the insoluble matter was subtracted from the weight of total ash and the difference in weight helps in determining the weight of the water soluble ash. The percentage of the water soluble ash was determined with reference to the air dried drug[18,19].

### Acid Insoluble Ash

The ash was boiled with 25 ml of dilute Hydrochloric acid for 5 minutes. The insoluble matter was collected on ash less filter paper (Whatmann Filter paper No.42). It was washed with hot water and the insoluble matter was incinerated along with filter paper in a previously weighted silica crucible not exceeding 450°C to a constant weight. The percentage of the acid insoluble ash was determined with reference to the air dried drug[18,19].

### Moisture Content

The moisture content of the drug was determined by Toluene distillation method (Dean and Stark Method), 10 gm of test drugs was taken in the flask of toluene distillation apparatus and 75 ml of distilled toluene was added and heated for subsequently for 5 hours. The volume of the water collected in the receiver tube (graduated in ml) was noted and the percentage of moisture content was calculated,[17,18] .

10 gm of powdered drug was taken, spread uniformly as a thin layer in a shallow petridish. It was heated at a regulated temperature of 105ºC, cooled in a desiccator and weighted. The process was repeated many times till two consecutive weights were found constant. Loss in weight was calculated with respect to initial weight in reference of percentage[17,20].

### Determination of pH value

Determination of pH was carried out by a synchronic digital pH meter (model no. 335) equipped with a combined electrode. The instrument was standardized by using buffer solution of 4.0, 7.0, and 9.20 to ascertain the accuracy of the instrument prior to the experiment.

### The pH value of 1% aqueous solution

An accurately weighted 1 gm of drug was dissolved in distilled water and the volume was adjusted accurately to 100 ml in a conical flask and allowed to stand overnight. It was filtered and the pH of 1% solution was measured with pH meter at particular temperature until two successive reading agree within +0.02 unit[18,19,20].

### The pH value of 10% aqueous solution

An accurately weighted 10 gm of drug was dissolved in distilled water and the volume was adjusted accurately to 100 ml in a conical flask and allowed to stand overnight. It was filtered and the pH of 10 % solution was measured with pH meter at a particular temperature until two successive reading agree within +0.02 unit[18,19,20].

**Bulk Density**

The tapped density is an increased bulk density attained after mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume readings are taken until little further volume change is observed. The mechanical tapping is achieved by raising the cylinder and allowing to it drop, under its own mass, a specified distance by either of manually methods or with the help of Apparatus. The bulk and tapped density are expressed in gm/ml, here ml and $cm^3$ are equivalent volume[21].

$$\text{Bulk Density} = \frac{\text{Wieght of the Powder drug /gm}}{\text{Volume of Cylender in cm3 or ml}}$$

**Determination of Extractive values**

The extractive values of all the test drugs in different solvent viz. Petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, ethanol, and distilled water were determined with the help of soxhlet,s apparatus (Successive method). The heat was applied for 6 hours on a heating mantle, after that it was evaporated on water bath till the weight become constant. The temperature of heating mantle and water bath was maintained according to the solvent used for the extraction. The extracts were filtered and after evaporation of the solvents, the extractive values were determined and percentage of extract was calculated with reference to the air dried drug. The procedure was repeated for three times and the mean value for each extract was calculated[19.20].

**Water and Alcohol Soluble Contents**

5 gm of the air dried powdered drug was taken with 100 ml of distilled water, in a glass stoppard conical flask for 24 hours. The mixture was carefully shaken frequently for 6 hours and then allowed standing for 18 hours. It was filtered and 25ml of filtrate was evaporated to dryness on a water bath. The residue was dried at 105°C to constant weight, cooled in desiccator for 30 minutes and weighed. The percentage of water soluble matter was calculated with reference to the amount of air dried drug. The percentage of alcohol soluble matter was determined as above by using alcohol in place of water[19].

**Phytochemical Analysis**

**Qualitative Analysis**

The qualitative analysis of different chemical constituents present in test drugs was carried out according to the scheme proposed by bhattacharjee[22]

The powder of the test drugs was extracted with petroleum ether (BP, 60-80°C). The petroleum ether extract (I) was tested for phenols, alkaloids and sterols/terpenes. A part of this extract was saponified and this portion (II) was tested for fatty acids, whereas, unsaponified portion (III) was tested again, phenols, and sterols/terpenes for confirmation. The defatted mark was divided into two portion, one portion was extracted with hot water and the other with ethanol (70%). The aqueous (IV) and ethanolic (V) extracts were tested for alkaloids, flavonoids, saponins, sugars, and tannins. Aqueous extract was extracted with ether, and ether soluble portion (VI) was tested again for alkaloids, sterols/terpene, whereas water soluble portion (VII) was tested for glycosides. The water soluble portion again hydrolysed with 5% hydrochloric acid and extracted with chloroform. The aglycone portion (VIII) was tested for insoluble hydrochloride of alkaloids. Chloroform soluble portion (IX) was tested for alkaloids and sterols / terpenes, whereas, water soluble fraction (X) was tested for alkaloids. One part of this water soluble portion was basified with any alkali (ammonia) and extracted with immiscible solvent (ether). The solvent soluble part (XI) was again tested for alkaloids.

**1. Test for alkaloids**

A drop of Dragendroff's reagent in the extract was added. The brown precipitate shows the presence of alkaloids[17]

**Hager' Test**

Few drops of Hager's reagent were added in 1 ml of alcoholic test solution. The presence of yellow colour precipitate indicates the presence of alkaloids.

**Wagner' Test**

Few drop of Wagner' reagents were added in 1 ml of alcoholic test solution dissolved with 2 ml of dil. HCl. The presence of yellow brown colour precipitation indicates the presence of alkaloids[17]

**2. Test for carbohydrate / sugars**

**I - Fehling's Test**

In the aqueous extract, a mixture of equal parts of Fehling's solution A and B previously mixed was added and heated. A brick red precipitate of cuprous oxide indicates the presence of reducing sugars.

**II - Molisch Test**

In an aqueous solution, α-napthol was added. Afterwards, concentrated sulphuric acid was gently poured. A brown colour ring at the junction of two solutions indicates the presence of the sugar[17]

**3. Test for Flavonoids**

A piece of magnesium ribbon was added to the ethanolic extract of the test drug followed by drop wise addition of concentrated HC1. Colour ranging from orange pink to red is a confirmatory test for flavonoids[23].

**4. Test for Glycosides**

The test solution is to be filtered and sugar is removed by fermentation with baker's yeast. The acid is removed by precipitation with magnesium oxide or barium hydroxide. The remaining alcoholic extract contains the glycosides was subsequently detected by the following methods,

**a.** The hydrolysis of the solution is to be done with concentrated sulphuric acid and after the hydrolysis sugar is determined with the help of Fehling's solutions.

**b.** The Molisch's test is done for sugar using α-napthol and concentrated sulphuric acid[17].

**5. Test for Tannin**

Ferric chloride solution was added in the aqueous extract of the drug. A bluish black colour which disappeared on addition of dilute sulphuric acid followed by a yellowish brown precipitate, shows the presence of tannin[17].

**6. Test for protein**

**Million's reaction**

To the test solution, Million's reagent was mixed and white coloured precipitate showed the presence of proteins.

**Biurette's Reaction**

In the hot test solution, 1 ml concentrated sodium hydroxide was added, followed by one drop of copper sulphate solution. A violet or red colour indicated the presence of proteins

**Xanthoproteinic Reaction**

In the test solution, concentrated nitric acid was added. A yellow precipitate appeared which dissolved in strong solution of ammonia and gave yellow colour, showing the presence of proteins[17].

**7. Test for Starch**

0.015 gm of Iodine and 0.015 gm of potassium Iodide was added in 5 ml of distilled water, 2 ml of iodine solution formed was added to 2 ml of aqueous test solution. The presence of blue colour indicates the presence of starch[22].

**8. Test for Phenol**

5-8 drops of 1% aqueous solution of lead acetate was added to aqueous or ethanolic test solution. The presence of yellow coloured precipitate indicates the presence of phenols[17].

**9. Test for Sterol / Terpenes**

**Salkowski reaction** In the test solution of chloroform, 2 ml concentrated sulphuric acid was mixed from the side of the test tube. The colour of the ring at the junction of the two layers was observed. A red colour ring indicates the presence of sterols / terpenes[17].

**10. Test for Amino Acids**

The ethanolic extract was mixed with ninhydrin solution (0.1% in acetone). After heating gently on water bath for few minutes, it gives a blue to red-violet colour that indicates the presence of amino acids[24].

**11. Test for Resin**

The test solution was gently heated and acetic anhydride was added in it. After cooling, one drop of sulphuric acid was mixed. A purplish red colour that rapidly changed to violet indicates the presence of resins[17].

**Test for Saponins**

**(a)** The defatted marc (0.5 gm) was boiled with water for 2 minutes in a test tube. After cooling, the mixture was vigorously shaken and then left for 3 minutes. The amount of honey comb frothing was classified as- no froth – negative; froth less than 1cm - weakly positive; froth greater than 1cm - highly positive; froth greater than 2 cm - strongly positive[22].

**(b)** The marc was boiled with water for 2 minutes. After cooling haemolysis test was performed. Haemolysis of blood indicated the presence of saponins[22].

**(B) Fluorescence Analysis**

**(I). Fluorescence Analysis of powdered drugs**

Fluorescence analysis of the powdered drugs were done for identification, the powdered drugs were treated with different chemicals and observed in day light and under ultra violet light. The changes in colour were noted.

**(ii). Fluorescence Analysis of the successive extracts of the test drugs**

Successive extracts of the test drugs viz. Petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, ethanol and aqueous extract were observed in day light and UV lights.

**(C) Thin Layer Chromatography (TLC)**

Thin Layer Chromatography of different extract was carried out on T.L.C. pre-coated aluminium plates (silica gel 60 of $F_{254}$ layer thickness 0.25 mm) by taking petroleum ether: diethyl ether in 1:1 ratio and n-butanol: Acetic acid: Water in 5:1:4 ratio as the mobile phase. The Rf values of the spots were calculated by the following formula[17,19].

$$R_f \text{ value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

## RESULTS AND DISCUSSION

Physicochemical standardization is a pre-requisite in quality control of natural drugs, both single as well as compounded drug. The efficacy of drug mainly depends upon its physical and chemical properties, therefore the determination of physicochemical characters and thereby the authenticity of drug is necessary before studying it for pharmacological activities. The techniques involved in the process of standardization encompass different parameter that together constitutes the profile of a drug. Determination of physicochemical properties also provides an index of purity and authenticity of the drug that in turn helps in quantifying the pharmacological effects and determination of the doses for various degrees of effects. Physicochemical study is also important because it helps in characterizing different constituents or group of constituents that frequently lead to establish the structure activity relationship and the likely mechanism of action of the drug. The percentage of different constituents also gives an idea of the magnitude and intensity of the effect of the drug. Apart from the degradation in the quality of the drugs that occurs due to climate, soil and processing condition, adulteration too contributes to its variability. Thus, the physicochemical study of the drug is a crucial aspect of the research study. The present study determines a comprehensive range of physicochemical characters of the drug according to the parameters used in pharmacopeia which may serve as the standard for ensuring optimum efficacy and safety of various samples of the drug. Sehjana **(***Moringa oleifera* Lam.**)** has been in use since times immemorial to treat wide range of indications. Present study deals with physicochemical and phytochemical investigation of dried fruit of Sehjana (*Moringa oleifera* L). The physicochemical investigation of the certain medicinal plant will be helpful for evaluation of nutritive value and preparation of Unani drugs and medicine. Phytochemical screening help to reveal the chemical nature of the constituents of Sehjana (*Moringa oleifera* Lam.) extract. Phytochemical analysis of extract showed that it contain alkaloid, steroids, phenol, amino acid, and terpenoids, carbohydrate and protein, were found in the extract (Table-3). The extractive values are a parameter for detecting the adulteration in any drug. The amount of the extracts that the drugs yield in a solvent is often an approximate measure of the amount of a certain constituent present in the drug. Therefore, for establishing the standard of any drug the extractive values play a major role (Table-2). Ash value is the residue that remains after complete incineration of the drug. Ash value plays an important role in ascertaining the standard of a drug, because the dust, earthy and un-required matters are generally added for increasing the weight of a drug resulting in the higher ash percentage. Therefore, the ash value determination furnishes the basis of judging the identity and cleanliness of a drug and give information

related to its adulteration with inorganic matter (Table-2). The percentage of solubility of powder drugs is also considered as an index of purity. Different percentage of alcohol varies with respect to soluble extractives, whereas, the drugs obtained from different source may produce different extractive values, extracted with the same concentration of alcohol (Table-2). Thin layer chromatography is one of the important techniques used for detecting the adulteration of the drugs. The various compounds present in the drug separate, depending on the affinity of mobile and stationary phases. The resolution of different kinds of chemical components is determined by using TLC and calculating the $R_f$ values after detecting the spots in order to standardize the drug for its identity. If the drug is adulterated there might be appearance of the other compounds as adulterant, in turn may increase the number of spots. On the other hand the exhausted or deteriorated drugs may loose the components and the number of spots appeared might be less. $R_f$ values of various spots appeared in different solvents system have been noted in day light, UV light and the treatment with iodine vapors, (Table-6).

## CONCLUSION

The present study has determined some crucial physicochemical characters of the test drugs by way of their standardization, so that, future studies may be carried out on samples found comparable on the basis of these characters, thereby, ensuring the reproducibility of the scientific study of these drugs.

Table 1: Organoleptic characters of powdered drugs

| S. No. | Parameter | Sehjana |
|---|---|---|
| 1. | Colour | Grayish yellow |
| 2. | Appearance | Fine |
| 3. | Odour | Agreeable |
| 4. | Taste | Slightly bitter |

Table 2: physicochemical study of powder of *Moringa oleifera Lam*

| S.NO | Parameters | Percentage (w/w)* |
|---|---|---|
| **1.** | **Ash value** | |
| | Total ash | 6.44±0.037 |
| | Acid insoluble ash | 3.94±0.026 |
| | Water soluble ash | 2.53±0.040 |
| **2.** | **Soluble Part** | |
| | Ethanol soluble | 15.2±0.91 |
| | Aqueous soluble | 22.93±0.48 |
| **3.** | **Successive Extractive Values** | |
| | Pet. Ether | 3.03±0.06 |
| | Di-ethyl ether | 1.3±0.05 |
| | Chloroform | 1.0±0.08 |
| | Acetone | 1.5±0.10 |
| **4.** | Alcohol | 13.1±0.25 |
| **5.** | Aqueous | 18.56±0.66 |
| **6.** | **Moisture content** | 8.6±0.33 |
| | **Loss on Drying** | 9.13±0.21 |
| **7.** | **pH values** | |
| | 1% water solution | 5.45±0.04 |
| | 10% water solution | 4.81±0.02 |
| | **Bulk density** | 0.33±0.00 |

*Note: Values are average of three experiments

Table 3: preliminary screening of major phytochemicals of Sehjana (*Moringa oleifera*)

| S.NO. | Chemical constituents | Tests/reagent | Inference |
|---|---|---|---|
| 1. | Alkaloid | Dragendroff´s reagent | +ve |
| | | Hager´s test | +ve |
| | | Mayer´s reagent | +ve |
| 2. | Carbohydrate | Molisch´s Test | +ve |
| | | Fehling´s test | +ve |
| 3. | Glycoside | NaOH Test | -ve |
| 4. | Flavanoids | Mg ribbon and Dil. Hcl | -ve |
| 5. | Tannin | Ferric chloride test | +ve |
| 6. | Protein | Xanthoproteic test | +ve |
| | | Biuret´s test | +ve |
| 7. | Sterol/Terpenes | Salkowski reaction | +ve |
| 8. | Amino acid | Ninhydrin solution | +ve |
| 9. | Resins | Acetic Anhydride Test | -ve |
| 10. | Phenol | Lead acetate Test | +ve |
| 11. | Saponin | Frothing with $NaHCO_3$ | -ve |

*Indications: „–ve" Absence and „+ve" presence of constituent.

Table 4: fluorescence analysis of extraction of *Moringa olifera*

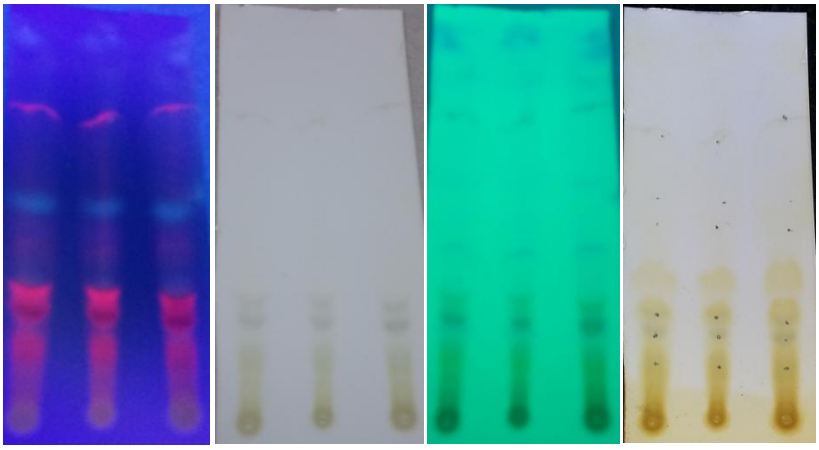
| S. No. | Extract | Day Light | UV Long | UV Short |
|---|---|---|---|---|
| 1 | Pet. Ether | Light Brown | Brown | Green |
| 2. | Di- Ether | Light Brown | Black | Light Green |
| 3 | Chloroform | Light Brown | Golden | Green |
| 4 | Acetone | Light Brown | Green | Light Green |
| 5 | Alcohol | Golden | Dark Green | Dark Green |
| 6 | Aqueous | Black | Dark Black | Dark Green |

Table 5: fluorescence analysis of powder drug Sehjana (*Moringa oleifera* lam)

| S. No. | Powder drug + Chemical Reagent | Day light | UV Short | UV Long |
|---|---|---|---|---|
| 1. | Powdered drug + Conc. $HNO_3$ | Orange | Green | Black |
| 2. | Powdered drug + Conc. HCl | Dark Brown | Dark Green | Black |
| 3. | Powdered drug + Conc.$H_2SO_4$ | Dark Brown | Black | Redish Black |
| 4. | Powdered drug + 2 % Iodine solution | Orange | Dark Green | Black |
| 5. | Powdered drug + Glacial Acetic Acid +$HNO_3$ | Light Brown | Green | Green |
| 6. | Powdered drug +Glacial Acetic Acid | Light brown | Light Green | Black |
| 7. | Powdered drug +NaOH (10%) | Brown | Green | Black |
| 8. | Powdered drug + Dil. $HNO_3$ | Light Brown | Green | Dark Green |
| 9. | Powdered drug + Dil. $H_2SO_4$ | Light Brown | Dark Green | Black |
| 10. | Powdered drug +Dil. HCl | Light Brown | Green | Black |
| 11. | Powdered drug +Dragendorff's | Greenish. B | Dark Green | Black |
| 12. | Powdered drug + Wagner's Reagent | Dark Brown | Dark Green | Black |
| 13. | Powdered drug + Benedict' Reagent | Greenish | Green | Dark Green |
| 14. | Powdered drug + Fehling Reagent | Brown | Green | Black |
| 15. | Powdered drug + KOH (10%) Methanol | Light Brown | Green | Black |
| 16. | Powdered drug + $CuSO_4$ (5%) | Light Green | Green | Black |
| 17. | Powdered drug +Ninhydrin (2%) in Acetone | Brown | Green | Black |
| 18. | Powdered drug + Picric Acid | Yellow | Green | Dark Green |
| 19. | Powdered drug + Lead Acetate (5%) | Light Brown | Light Green | Black |

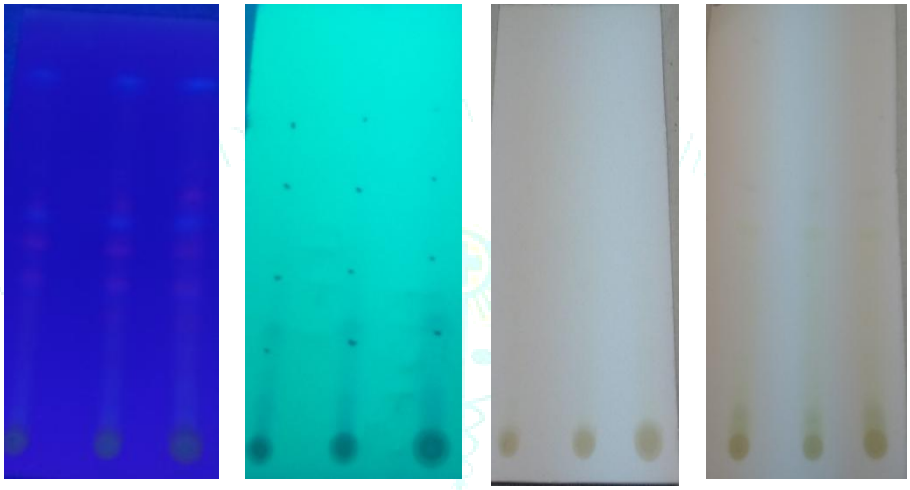
Table 6: thin layer chromatography of Sehajan (*Moringa oleifera* lam)

| Treatment | Mobile Phase: | No of Spots | Rf value and colour of spots |
|---|---|---|---|
| **Petroleum Ether Extract** | | | |
| Day light | Petroleum ether: Di-ethyl ether (4:1) | 4 | 0.20 (green), 0.27 (dark green), 0.32 (green), 0.75 (green). |
| UV short | | 5 | 0.20 (green), 0.27 (dark green), 0.32 (green), 0.44 (green), 0.75 (dark green). |
| UV long | | 6 | 0.20 (pink), 0.27 (red), 0.32 (pink), 0.53 (blue), 0.60 (blue), 0.75 (pink). |
| Iodine vapor | | 5 | 0.20 (golden), 0.27 (brown), 0.32 (orange), 0.38 (orange), 0.75 (black). |
| **Chloroform Extract** | | | |
| Day light | Petroleum ether: Ethanol (4:1) | 2 | 0.24 (light green), 0.39 (green). |
| UV short | | 4 | 0.24 (green), 0.39 (green), 0.57 (black), 0.67 (green). |
| UV long | | 4 | 0.24 (pink), 0.39 (pink), 0.48 (blue), 0.57 (blue). |
| Iodine vapor | | 3 | 0.24 (brown), 0.39 (yellow), 0.57 (yellow). |
| **Di-ethyl Ether Extract** | | | |
| Day light | Petroleum ether: Di-ethyl ether (4.1) | 3 | 0.20 (brown), 0.36 (green), 0.83 (light green). |
| UV short | | 5 | 0.20 (green), 0.36 (dark green), o.44 (green), 0.55 (black), 0.71 (green). |
| UV long | | 6 | 0.20 (blue), 0.36 (pink), 0.44 (red), 0.48 (blue), 0.52 (pink), 0.83 (blue). |
| Iodine vapor | | 4 | 0.20 (green), 0.36 (brown), 0.48 (dark green), 0.83 (brown). |



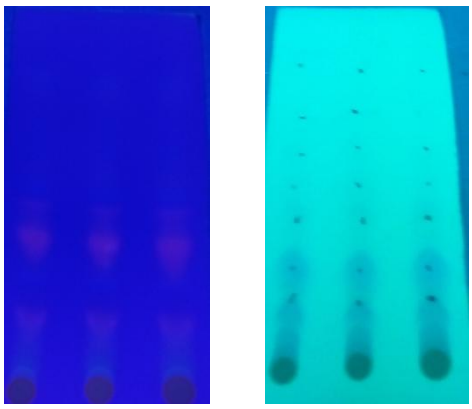
UV Long Day light UV short Iodine vapor

Figure 1: TLC of Petroleum ether Extract of Sehjana(*Moringa oleifera* Lam).



UV long UV short Day light Iodine vapor

Figure 2: TLC of Diethyl ether extract of Sehjana (*Moringa oleifera* Lam).



UV long UV short

Figure 3: TLC of Chloroform extract of Sehjana (*Moringa oleifera* Lam).

**Conflict of interest statement:** We declare that we have no conflict of interest.

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